ON THE FORMATION OF HEMOLYMPH NODES DURING THE ALARM REACTION

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FOUR FIGURES

The object of this communication is to report on a series of observations showing that hemolymph nodes may be formed from ordinary lymph nodes within a few hours, during the 'alarm reaction.'

PREVIOUS INVESTIGATIONS ON THE ALARM REACTION

It has been shown that exposure to various nocuous agents such as cold surrounding temperature, trauma, nervous injuries, toxic doses of various drugs, or excessive muscular exercise elicits a syndrome which has been regarded as the response of the organism to damage as such. Since this syndrome is only obtained during the first 24 to 48 hours after the organism is first confronted with the damaging agent and not after subsequent exposures, it has been considered as the somatic expression of the alarm caused by a stimulus to the quality or intensity of which the organism is not adapted. Until we learn more about the mechanism of this reaction, we shall simply refer to it, therefore, by the non-committal term the 'alarm reaction' (Selye, '36 a, '36 b).

The most characteristic morphological changes observed during this reaction are: the rapid involution of the thymus and other lymphatic organs, hypertrophy of the adrenal cortex, loss of chromaffinity of the adrenal medulla formation of edema and pleural transudate, formation of multiple ulcers and hemorrhages in the gastro-intestinal tract (Selye, '37 a,

'37 b) and neutrophilic leukocytosis with relative lymphopenia (Harlow and Selye, '37). Under certain circumstances, when the reaction is particularly acute and severe, one may also see clouding of the crystalline lens (Selye, '37 b) and specific lesions in the appendix (Selye, '37 c), but the latter symptoms are inconstant.

Among the chemical changes characteristic of the alarm reaction, a transitory increase in blood sugar followed by hypoglycemia and an initial hypochloremia with a subsequent rise of the blood chlorides above the initial level have been found to be fairly constant (Selye, '37 b; '38 a).

It has also been shown that the alarm reaction may be regarded as helpful for the defence of the organism against nocuous agents. Animals in which an alarm reaction has been elicited by a certain stimulus become more resistant not only against this stimulus but also against agents of a different nature (Selye, '37 d, '37 e, '38 b, '38 c).

It has been demonstrated furthermore that the excretion of water given during the alarm reaction is greatly delayed (Howlett and Browne, '37). Using this as an indicator, one may also show an increase in non-specific resistance during the alarm reaction. If during the course of this reaction produced by any stimulus, the same or any other stimulus—which proves capable of eliciting an alarm reaction in not pretreated animals—is applied, the second stimulus not only fails to cause water retention but actually increases urine output (Karády et al., '38).

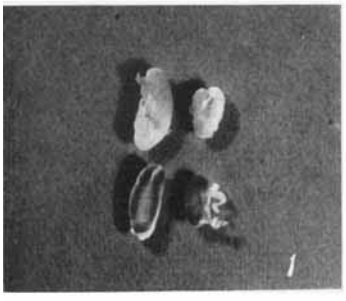
EARLIER STUDIES ON THE EXPERIMENTAL PRODUCTION OF HEMOLYMPH NODES

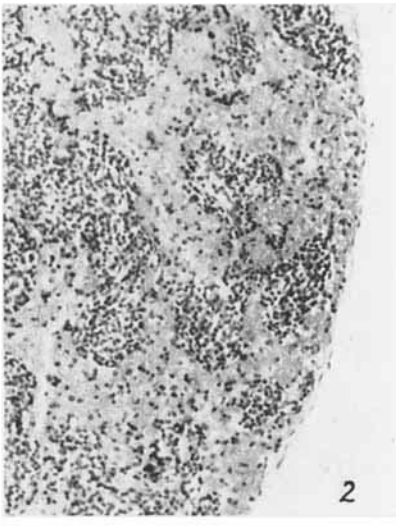
Warthin ('09, '16) and Meyer ('14) claimed that splenectomy does not produce hemolymph nodes in the dog. In this respect, they contradict the earlier findings of Tizzoni (quoted by Meyer). On the other hand, ligature of the splenic vein forms hemolymph nodes as shown by Warthin ('09) and Jäeger ('31, '37) but only in the vicinity of the hilum of the spleen. Dawson ('27) in dogs and Michels ('35) in rabbits

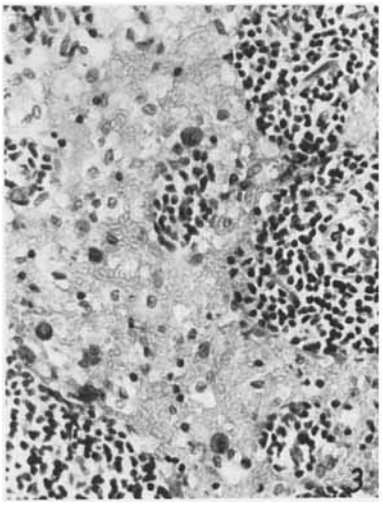
obtained formation of hemolymph nodes following x-ray irradiation of the abdomen. They think that such a treatment blocks the lymphatics as a result of which circulatory disturbances ensue which led to this transformation. Retterer ('02 a, '02 b) in cats, guinea pigs, rabbits and birds, claimed to have obtained transformation of ordinary lymph nodes into hemolymph nodes within a few hours by simultaneously fasting and bleeding his experimental animals. The author thinks that the resulting decrease in the blood pressure of these badly damaged animals would prevent the removal of red cells from their lymph nodes, so that the erythrocytes accumulate in the sinuses. This argument hinges on his assumption that these nodes form red cells. When cantharidin was injected locally into the leg of a guinea pig or rabbit, the regional lymph nodes were transformed into hemolymph nodes while the other nodes showed no change. Dudgeon et al. ('09) treated their experimental animals with so-called 'splenotoxic' and hemolitic sera, and claimed to have obtained hemolymph nodes as well as all the transitional stages between these and ordinary lymph glands. It is evident from their description that their animals, which showed adrenal enlargement, gastro-intestinal hemorrhages, thymus atrophy etc., responded to these sera with a pronounced alarm reaction although the investigators failed to recognize this. It is likewise most probable that the drastic measures employed by the previously mentioned investigators (x-ray treatment, severe hemorrhages, cantharidin treatment) were also capable of producing an alarm reaction. The same applies to the experiments of Meek ('10) who introduced living pathogenic bacteria into the peritoneum of guinea pigs or injected them with diphtheria toxin, oxalic acid, or distilled water and observed hemolymph node formation within 12 hours.

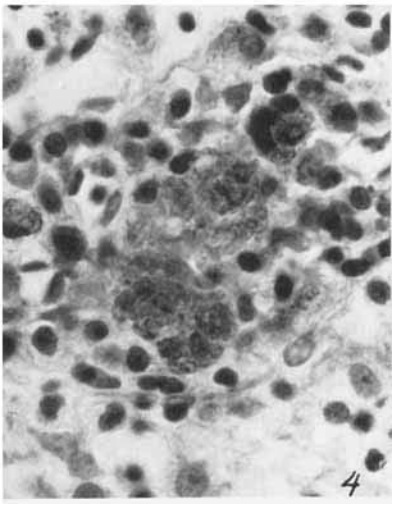
OBSERVATIONS SHOWING THE FORMATION OF HEMOLYMPH NODES DURING THE ALARM REACTION

Eighteen female rats weighing 172 to 186 gm. were used for the first experiment. In order to produce an alarm reaction, we exposed six of them to a temperature of +1°C.









Figures 1 to 4

for 3 hours. Another group of six was treated with three injections of 0.5 cc. of a 4% formaldehyde solution within 3 hours, while the remaining six were forced to perform intense muscular exercise for 2 hours, in revolving cages having a diameter of 12 inches and turning at a speed of 18 to 20 revolutions per minute. All these animals were killed 3 hours after the initiation of treatment. At this time, hemolymph node formation was quite evident, though not very pronounced, in the exercised group but only traces of blood were visible in the lymph nodes of the animals treated with formaldehyde or cold. This was easily detectable by simple naked eye inspection, inasmuch as most of the lymph nodes, especially the iliac, lumbar and axillary nodes, were bright red, particularly in the periphery, that is to say, in the region of the subcapsular sinus. Microscopical examination showed that the sinus was filled with erythrocytes (figs. 1, 2 and 3). It should be emphasized that the sinuses of these particular lymph nodes never contain red cells in the normal rat. As has been mentioned in the previous section, the lymph nodes of the thymus, the pancreas and the renal lymph nodes of the rat may normally show certain characteristics reminiscent of hemolymph nodes. This is especially true of the pancreatic and renal nodes, which are invariably brown in the adult rat. The dark brown color of these normal glands, however, differs distinctly from the bright red hemolymph nodes seen immediately following the production of an alarm reaction. Histologically, the difference is still more obvious because the normal renal and pancreatic 'hemolymph nodes' contain no

Fig. 1 Microscopical picture of two iliac lymph nodes of a normal rat (on top) and of the two corresponding lymph nodes of a rat in which an alarm reaction has been produced by excessive muscular exercise (below). The appearance of erythrocytes in the peripheral sinus accounts for the characteristic dark rim surrounding the hemolymph nodes.

Fig. 2 Low magnification of the peripheral part of an experimentally produced hemolymph node similar to that shown in figure 1. Note the blood in the sinuses.

Fig. 3 High magnification of a part of the section shown in figure 2 in which the erythrocytes are easily distinguished.

Fig. 4 High magnification of a section through the adrenal 'hemolymph node' of a normal adult rat showing large phagocytes in the reticulum which are filled with iron pigment.

blood but only blood pigment-storing phagocytes in their sinuses and reticulum. This pigment, which has a greenish-brown color in unstained sections, is obviously an iron pigment since it gives a positive Prussian blue reaction, as we have been able to ascertain in numerous normal adult rats of our colony (fig. 4). In immature animals, these 'hemolymph nodes' are rarely if ever observed.

In our previous experiments on the effect of the alarm reaction on the excretion of intravenously administered physiological sodium chloride solutions (Karády et al., '38), we noticed that hemolymph node formation was especially marked. It appeared that if the blood volume is greatly increased by the intravenous administration of large quantities of fluid, hemolymph node formation is particularly pronounced. In order to establish this fact with greater certainty, we injected eighteen female rats, weighing 171 to 192 gm. intravenously with an amount of 0.85% NaCl solution corresponding to 10% of their body weight. After this, the animals were divided into three groups of six and an alarm reaction was produced by exposure to cold in the first, injection of formaldehyde in the second and muscular exercise in the third group, under conditions exactly identical with those of the first experimental series. A group of six females weighing 179 to 184 gm. was also injected with saline but not otherwise treated. Three hours after initiation of treatment in the first three groups, all the twenty-four rats were killed. Autopsy revealed marked hemolymph node formation in all the three groups in which an alarm reaction was produced but not in the control group, treated with saline only. The hemolymph node formation caused by the alarm reaction in these saline treated animals was much more generalized and pronounced than in the previous series. In addition to the formation of these hemolymph nodes, there were numerous hemorrhages in the thymus of all these animals and in some cases, the entire thymus assumed a bright red color similar to that of hemolymph nodes. From these experiments, it appears that although the intravenous administration of large

amounts of an isotonic NaCl solution does not cause hemolymph node formation itself, it sensitizes the rat to the hemolymph node forming action of alarming stimuli.

In order to establish whether these hemolymph nodes, once they are formed, are permanent structures or whether they disappear again after some time, we took two female rats, weighing 180 gm. each, injected them with 18 cc. of 0.85% NaCl intravenously and then forced them to run in the above-mentioned revolving cages twice for 90 minutes with a rest period of 3 hours in between. Immediately following this, one animal was killed and was found to have numerous bright red hemolymph nodes. Upon histological examination, the structure of these nodes differed somewhat from what we have seen in the first series, inasmuch as most of the erythrocytes in the sinuses of the nodes were engulfed in phagocytes showing the picture of the so-called 'erythrophagocyte' referred to by some investigators as the 'Russell body cell.' Beginning decomposition of the phagocytosed erythrocytes revealed itself by the appearance of a positive Prussian blue reaction. It appears that in this animal which was allowed to live longer after initiation of treatment than the animals in the previous series, destruction of the erythrocytes in the sinuses had already begun. This was even more marked in the second rat of this group which was killed 3 days later. In it, the peripheral sinuses of the lymph nodes were filled with large phagocytes but only few of them contained traces of green iron pigment. Most of the red cells and blood pigment containing phagocytes had disappeared and free red cells were entirely absent from the sinuses. This experiment showed that the appearance of red cells in the sinuses of lymph nodes during the alarm reaction is a transitory phenomenon and the removal of these cells begins within a few hours and is almost complete 3 days after their entrance into the lymphatic sinuses.

While most of our experiments were performed on the rat, we wish to emphasize that hemolymph node formation has also been seen in the guinea pig, rabbit and cat under the influence of various alarming stimuli so that this response is not a peculiarity of the rat only but it is common to numerous laboratory animals.

SUMMARY AND DISCUSSION

The above-mentioned findings show that lymph nodes containing phagocytes filled with hematogenic iron pigment are normally present in the vicinity of the adrenal (one on each side) and in the pancreas of the adult rat. Occasionally, such nodes are also observed in the thymus region. Their dark brown color makes it easy to detect them by naked eye inspection. They are rarely, if ever, observed in young prepubertal rats. These lymph nodes do not contain free blood under normal conditions.

Under the influence of the alarm reaction, red cells appear in the sinuses of various lymph glands in the rat and other experimental animals. The formation of these 'hemolymph nodes' is greatly facilitated when the blood volume is increased by the intravenous infusion of fluid. The invasion of red blood cells during the alarm reaction is not limited in the rat to those lymph nodes which normally contain hematogenous pigment, but is generalized. The red cells which appear in the sinuses are soon engulfed by phagocytes which digest and remove them within a few days.

It is clear from our description that neither the normal nor the experimentally produced 'hemolymph nodes' of the rat have the characteristics postulated by some investigators, for their sinuses do not contain normal blood and do not communicate freely with blood vessels. If one accepts these criteria as the characteristics of hemolyph nodes, then the rat normally has no hemolymph nodes and even during the alarm reaction, it is only during the first few hours that its lymph nodes could be regarded as true hemolymph nodes according to this definition. It might be preferable, therefore, to refer

to these nodes as 'iron pigment lymph nodes.' Whether these iron pigment lymph nodes are only one type of the hemolymph nodes is questionable as yet. It is evident however, from the histological description of hemolymph nodes given by various authors, that they actually dealt with structures identical with those which we observed, in certain regions, in the normal rat and which we produced experimentally in other regions by eliciting an alarm reaction.

Nothing definite can be said as yet about the physiological significance of these iron pigment lymph nodes, but their appearance during the alarm reaction suggests that they may be of some importance for the defence of the organism against damaging stimuli. These findings together with the fact mentioned above, that such structures have also been observed to appear both in animals and in man as a result of naturally occurring diseases, makes it very likely that they play a more important role in pathology than has hitherto been suspected.

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